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Role of HLA class II genes in susceptibility and resistance to multiple sclerosis: studies using HLA transgenic mice

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Abstract

Multiple sclerosis (MS), an inflammatory and demyelinating autoimmune disease of CNS has both, a genetic and an environmental predisposition. Among all the genetic factors associated with MS susceptibility, HLA-class II haplotypes such as DR2/DQ6, DR3/DQ2, and DR4/DQ8 show the strongest association. Although a direct role of HLA-DR alleles in MS have been confirmed, it has been difficult to understand the contribution of HLA-DQ alleles in disease pathogenesis, due to strong linkage disequilibrium. Population studies have indicated that DQ alleles may play a modulatory role in the progression of MS. To better understand the mechanism by which HLA-DR and -DQ genes contribute to susceptibility and resistance to MS, we utilized single and double transgenic mice expressing HLA class II gene(s) lacking endogenous mouse class II genes. HLA class II transgenic mice have helped us in identifying immunodominant epitopes of PLP in context of various HLA-DR and -DQ molecules. We have shown that HLA-DR3 transgenic mice were susceptible to PLP₉₁₋₁₁₀ induced experimental autoimmune encephalomyelitis (EAE), while DQ6 (DQB1*0601) and DQ8 (DQB1*0302) transgenic mice were resistant. Surprisingly DQ6/DR3 double transgenic mice were resistant while DQ8/DR3 mice showed higher disease incidence and severity than DR3 mice. The protective effect of DQ6 in DQ6/DR3 mice was mediated by IFN γ , while the disease exacerbating effect of DQ8 molecule was mediated by IL17. Further, we have observed that myelin-specific antibodies play an important role in PLP₉₁₋₁₁₀ induced EAE in HLA-DR3DQ8 transgenic mice. Based on these observations, we hypothesize that epistatic interaction between HLA-DR and -DQ genes play an important role in predisposition to MS and our HLA transgenic mouse model provides a novel tool to study the effect of linkage disequilibrium in MS.

Keywords

EAE/MS; HLA transgenic mice; cytokine; anti-myelin antibody; complement

1. Introduction

Multiple sclerosis (MS) is presumed to be an autoimmune disease of the central nervous system (CNS) leading to demyelination, axonal damage, and progressive neurologic disability. Collective evidence suggests that the onset of the disease might result from an

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aberrant immune response to a number of myelin antigens that is T-cell mediated. The first process of autoimmunity is the peripheral activation of auto-reactive CD4⁺ T-cells via the presentation of auto-antigens by susceptible MHC class-II molecule(s). Therefore it is not surprising that autoimmune diseases such as MS show a strong association with certain HLA class II genes [1-8].

The HLA class II region of the MHC on chromosome 6p21 accounts for the majority of familial clustering in MS and is by far the major susceptibility locus. The class II linkage in MS differs in various populations with the highest association with HLA-DR2 (DRB1*1501)/DQ6 (DQB1*0602) [9-12]. Elegant studies by Dyment et al [4] have shown that the DRB1*17 (DR3) allele is also associated with MS susceptibility. A similar finding on the association of DR3 with MS has been shown in Southern European, Canadian, Mexican and Sardinian MS patients [1, 13-15]. Beside DR2/DQ6, DR3/DQ2 and DR4/DQ8 genes are also linked with predisposition to MS [1, 12, 14, 16-18]. Recent studies have shown that disease outcome might be decided by a complex interaction among different class-II genes present in a 'haplotype', suggesting that the 'haplotype' might be the basic immunogenetic unit of susceptibility or resistance [3, 4, 7, 8, 19].

Although no animal model can mimic all the facets of human MS, the experimental autoimmune encephalomyelitis (EAE) model in rodents has helped immensely in improving our understanding of the immunopathogenesis of MS [20-22]. EAE can be induced in various inbred animal strains by inoculation of whole myelin or defined myelin proteins such as myelin basic protein (MBP), myelin oligodendrocytes glycoprotein (MOG), and proteolipid protein (PLP) in complete Freund's adjuvant [20-22]. Elegant studies in murine/rodent EAE have documented that encephalitogenic T cells are CD4⁺, T helper (Th1)-type cells secreting TNF- α / β and IFN γ [23-25]. However recent studies have indicated that a new T cell phenotype Th17 secreting IL-17, IL-17F, IL-21, IL-22 and IL-23 might also play an important role in the immuno-pathogenesis of EAE [26]. Thus current hypothesis of EAE indicates that both Th1 and Th17 cytokines play important roles in the immunopathogenesis of EAE.

2. HLA Class II Transgenic Mice Expressing HLA-DR or -DQ Molecule as an Animal Model of MS

Despite the fact that MHC genes show the strongest association with MS, the exact role of HLA-DQ and -DR genes in disease pathogenesis is not well understood due to the high polymorphism and heterogeneity of human populations. The strong linkage disequilibrium among HLA-DR, -DQ and other genes within the HLA region makes it difficult to identify the role of individual genes in the immunopathogenesis of MS. In order to understand the role of class-II molecules in MS, transgenic mice were generated that express human HLA-DR or -DQ genes lacking endogenous mouse class II genes [27]. A EAE mouse model where the autoreactive T cell repertoire is selected and shaped by human MHC class II molecules has helped us in understanding the immunopathogenesis of inflammatory and demyelinating diseases such as human MS. Using these class II transgenic mice, first we tested whether these human HLA class II molecules are functional by analyzing the T cell proliferative response against various CNS antigens.

2.1. Identification of T cell epitopes

We carried out experiments to determine whether HLA class II molecules in the transgenic mice can efficiently present myelin antigen proteolipid protein (PLP). PLP is the most abundant myelin antigen in CNS and T cells reactive to PLP peptides had been identified in both MS patients and normal controls [28-30]. Using overlapping PLP peptides

encompassing the entire sequence of the human PLP molecule (human and mouse PLP are 100% conserved), we identified a number of epitopes restricted to various HLA-DR or -DQ molecules (Table 1). T cell epitopes were spread throughout the entire sequence of PLP molecule [31] and major immunodominant regions were 31-70, 81-120, 140-160, 178-227 and 264-277. Both HLA-DR2 (*1502) and HLA-DR4 (*0401) molecules recognized similar residues on the PLP protein encompassing residues 31-60, 81-120, 178-197, 208-227 and 264-277. The exceptions were PLP₅₁₋₇₀, recognized only by -DR4 molecule, and residue 198-207 recognized only by -DR2 molecule. HLA-DR3 molecules recognized residues 41-60, 91-110, and 178-227. In summary, all -DR (DR2, DR3 and DR4) and -DQ (DQ6 and DQ8) molecules, recognized regions 41-60, 91-110, 178-197 and 208-227 of PLP. The majority of epitopes identified largely encompassed regions previously reported to be immunogenic in humans [28-30, 32]. More importantly not all epitopes were restricted to various class II molecules tested. While, some peptides elicited a response specific to a particular HLA class II allele, others were promiscuous. PLP₁₃₉₋₁₅₄ was immunogenic in DQ6 and DQ8 transgenic mice but not in DR2, DR3 and DR4 transgenic mice. PLP peptide 1-20 was immunogenic only in DQ8 mice. In addition, we observed both DQ and DR restricted response to PLP₉₁₋₁₁₀ whereas responses to 264-277 residues of PLP were restricted only to DR molecule. A similar observations have also been reported in a study of MS patients from Japan [32]. Thus HLA class II transgenic mice authenticate restriction of the proteolipid protein (PLP) specific immune response implicated in MS pathogenesis.

2.2. PLP induced EAE in HLA-class II transgenic mice

Our T cell epitope mapping data identified four immunodominant PLP epitopes (PLP 41-60, 91-110, 178-197, and 208-227) in all DR and DQ transgenic mice. Previously, PLP₉₅₋₁₁₆ had been shown to be restricted by HLA-DR and -DQ using T cell lines from MS patients while PLP₉₅₋₁₁₆ specific T cell clones from HLA-DR2 transgenic mice can induce EAE in Rag2^{-/-} mice. These results support a pathogenic role for PLP₉₅₋₁₁₆ specific T cells in HLA-DR2⁺ MS patients, and shed light on the possible correlation between autoimmune target epitope and disease phenotype in human CNS autoimmune diseases [33].

Based on these studies, we selected PLP₉₁₋₁₁₀ peptide for induction of EAE in HLA class II transgenic mice. PLP₉₁₋₁₁₀ peptide induces a progressive, chronic EAE in 67% of the HLA-DR3.Aβ° mice [31] and disease was characterized by a typical course of ascending paralysis. The mean onset of the disease was 16±3 days, and maximum disease severity score ranged from 1-3. No clinical sign of disease was seen in transgene negative controls. The majority of affected DR3 mice never went into remission for the length of the test period (10 weeks), and developed a chronic form of EAE. No clinical symptoms were observed in HLA-DR2, -DR4 or HLA-DQ6 or -DQ8 transgenic mice [31]. DR3 transgenic mice with clinical signs of EAE had diffuse meningeal infiltrates in both the spinal cord and the brain. In addition, occasional sections of the spinal cord showed paragonal mononuclear cell infiltrates that were closely associated with the meningeal infiltrates. In the brain, mononuclear cell infiltrates were seen primarily in the meningeal surfaces of the brain stem, cerebellum, and surrounding the ventricles. Small areas of demyelination were observed in spinal cord of DR3 mice with EAE.

Next we examined whether induction of EAE by PLP₉₁₋₁₁₀ could lead to intra-molecular or inter-molecular spread of T cell responses to other PLP regions or CNS antigens. Beside PLP₉₁₋₁₁₀ induced proliferation responses, T cell proliferation was detected against PLP peptides 141-160, 170-197, 188-207, 208-227, and recombinant MOG, but not MBP protein. Using single amino acid truncation and alanine substitution of encephalitogenic PLP₉₁₋₁₁₀, we identified the minimal epitope necessary for binding to the DR3 molecule and to induce EAE [34]. Residues necessary for binding to HLA-DR3 molecule were identified as amino acid 97-108 of PLP. Immunization of DR3 transgenic mice with the minimal epitope

PLP₉₇₋₁₀₈ led to induction of EAE and these mice showed classical pathology associated with EAE. The alanine substitutions study showed that residues 99, 102, and 103 are critical for immune recognition of HLA-DR3 molecule [34].

Beside PLP₉₁₋₁₁₀ peptide, Ito et al [35] showed that PLP₁₇₅₋₁₉₂ can induce a strong proliferative response and EAE in HLA-DR4 transgenic mice. Recently using a MBP-PLP fusion protein (MP4) [36], we have identified that PLP₁₇₈₋₁₉₇ peptide can induce EAE in HLA-DR2 (*1502) transgenic mice (Mangalam et al unpublished observation). Our observation along with previous studies indicate that presence of HLA-DR molecule is required for susceptibility to EAE, as transgenic mice expressing either the human DQ6 or DQ8 genes do not develop disease [27]. Based on these observation, we propose that HLA-DR genes such as HLA-DR2, -DR3 and -DR4 are responsible for predisposition and susceptibility to demyelinating disease, while polymorphism in DQ gene(s) might play a modulating role. This hypothesis is supported by population studies showing that the epistatic interaction between HLA molecules of the disease susceptible haplotypes plays an important role in the final disease outcome in MS. While HLA-DQB1*0601 and -DQB1*0603 protect against MS [3, 4, 19, 37, 38], DQB1*0602 and DQB1*0302 alleles can increase disease susceptibility [5, 11, 37, 39, 40]. To understand the role of HLA-DQ molecules in the disease process, we generated double transgenic mice expressing HLA-DQ6 or HLA-DQ8 gene on a disease susceptible HLA-DR3 background.

3. HLA-DQ6 (DQB1*0601) suppress EAE in HLA-DR3 transgenic mice by generating anti-inflammatory IFN- γ

Human population studies have suggested that HLA-DQ6 (DQB1*0601), found mostly in Asian populations protects from MS [37, 41]. To test this protective effect of DQ6 in an experimental model, we generated double transgenic mice expressing both HLA-DR3 and DQ6 on mouse class II negative background. Administration of PLP₉₁₋₁₁₀ to DR3.DQ6.A β^0 double transgenic mice with PLP₉₁₋₁₁₀ led to disease development only in 40% of double transgenic mice as compared to 70% disease incidence in parental DR3.A β^0 transgenic mice indicating, a protective role of the DQ6 gene [42]. The onset of disease between these two groups was similar. Transgene negative littermates or control A β^0 mice and DQ6 transgenic mice did not develop clinical disease. This clinical disease data suggested that DQ6 plays a protective role by inhibiting development of EAE in disease susceptible DR3 transgenic mice.

Next we analyzed if the protective effect of DQ6 is due to defect in ability of DQ6 molecule to recognize PLP₉₁₋₁₁₀ antigen. Interestingly, DR3.DQ6.A β^0 mice showed a very strong, dose dependent T cell response to PLP antigen, which were at least three to four folds higher in magnitude as compared to disease susceptible DR3.A β^0 mice. Similar to double transgenic mice, HLA-DQ6 transgenic mice also showed a very strong T cell proliferative response to PLP₉₁₋₁₁₀ as compared to DR3.A β^0 transgenic mice indicating that the DQ6 molecule can present PLP₉₁₋₁₁₀ antigen better than DR3.A β^0 transgenic mice. Using the antibody blocking experiment we confirmed that the strong T cell response observed in DR3.DQ6.A β^0 transgenic mice was restricted to DQ molecule. As EAE was considered to be Th1 mediated disease, we argued that resistance to EAE in DQ6 mice might be due to expression of Th2 cytokines, which had been shown to be protective in EAE. However, we observed that both the disease resistant DQ6 and protected DR3.DQ6.A β^0 transgenic mice produced high levels of IFN γ , a cytokine normally associated with development of EAE [43, 44]. Beside high levels of IFN γ , DR3.DQ6.A β^0 double transgenic mice also produced a moderate level of IL-10 and high levels of IL-2 and IL-27. IL-4 levels were below detection limits in all samples from single and double transgenic mice. In contrast, T cells from disease susceptible DR3.A β^0 transgenic mice produced higher levels of IL-17, IL-22, and

IL-23 as compared to DQ6 and DR3.DQ6 mice. We performed an IFN γ -Elispot in the presence or absence of blocking antibodies to confirm that HLA-DQ restricted CD4⁺T cells were the source of IFN γ and not CD8 T cells or NK cells.

Our cytokine data indicated that DQ6 restricted IFN γ might be responsible for the protective effect observed in DR3.DQ6.A β^0 transgenic mice. To confirm the role of IFN γ in disease protection, we performed in-vivo studies using neutralizing IFN γ antibody. DR3DQ6 transgenic mice treated with anti-IFN γ but not with isotype control showed increased disease incidence and severity, similar to DR3.A β^0 transgenic mice, confirming a protective role of IFN γ in this model of EAE. Neutralizing antibody treatment in DQ6.A β^0 mice had no effect. Thus high level of IFN γ plays an anti-inflammatory role and can suppress disease.

IFN γ shows its anti-inflammatory effect through various pathways such as induction of nitric oxide, generation of induced Tregs and apoptosis of antigen specific T cells. PLP₉₁₋₁₁₀ specific CD4 T cells from DQ6 mice and DR3DQ6 transgenic mice produced higher level of nitric oxide and had an increased frequency of CD4⁺FoxP3⁺ T cells as compared to disease susceptible HLA-DR3 restricted CD4 T cells. We also observed that T cells from DQ6 as well as DR3.DQ6.A β^0 mice undergo increased proliferation and apoptosis as compared to DR3 specific T cells. Thus the protective effect of DQ6 in DR3.DQ6.A β^0 double transgenic mice, was due to high levels of IFN γ produced by DQ6 restricted T cells, which suppressed proliferation of encephalitogenic DR3-restricted T cells by inducing apoptosis. Our study suggests that DQ6 modifies the PLP₉₁₋₁₁₀ specific T cell response in DR3 through the anti-inflammatory effects of IFN γ [42].

4. HLA-DQ8(DQB1*0302)Exacerbate Disease in HLA-DR3 mice by generating pro-inflammatory IL-17

Presence of the HLA-DQ8/DR4 haplotype has been associated with susceptibility to MS [16-18, 45]. To test the role of DQ8 (DQB1*0302) in the immunopathogenesis of MS, we generated HLA class II transgenic mice that express HLA-DQ8 and EAE susceptible DR3 on an MHC II deficient background. Administration of PLP₉₁₋₁₁₀ to DR3.DQ8.A β^0 double transgenic mice with led to development of disease in 100% of animals as compared to 70% disease incidence in parental DR3.A β^0 transgenic mice, indicating that DQ8 synergizes with DR3 for increased disease penetration. DR3DQ8 double transgenic mice showed an earlier disease onset with increased severity as compared to DR3 transgenic mice (mean clinical score 3.4 $\pm$ 0.2 vs. 2.3 $\pm$ 0.3, p<0.5). Thus DQ8 plays a modulatory role in DR3.DQ8.A β^0 double transgenic mice by inducing more severe EAE in disease susceptible DR3 transgenic mice [46].

Disease susceptible DR3.A β^0 transgenic mice produced moderate to high levels of IFN γ , TNF α , IL-2, IL-6, and IL-12 cytokines, showing classical Th1 phenotype. Although CD4 T cells from DQ8 mice did not produce IFN γ , they produced significantly higher levels of IL-17 and GM-CSF (p<0.01). Double transgenic DR3.DQ8 mice also produced higher levels of IL-17 and GM-CSF as well as IFN γ , besides producing moderate to high levels of TNF α , IL-1, IL-6, and IL-12 cytokines. IL-4 levels were below detection limits in all samples from single and double transgenic mice. DR3.A β^0 transgenic mice also produced moderate amounts of IL-17, IL-21, and IL-23, however, levels were significantly less (p<0.01) as compared to DQ8.A β^0 or DR3.DQ8.A β^0 mice. Both DR3 as well as DR3.DQ8.A β^0 transgenic mice produced comparable levels of IL-27. Thus, disease susceptible DR3.DQ8.A β^0 mice produced higher levels of IL-17, IL-21, IL-23, and IFN γ as compared to DR3 mice.

Using antibody blocking and cytokine Elispot assay, we confirmed that increased levels of IFN γ was produced by DR3 specific T cells, while IL-17 was produced by DQ8 specific T cells. To confirm the respective role of each cytokine in the EAE model, we neutralized either IL-17 or IFN γ in DR3.DQ8.A β^0 mice using specific blocking antibodies and their respective isotype controls. While neutralization of in-vivo levels of IFN γ showed no effect on disease incidence or severity, treatment with neutralizing IL-17 by its specific antibody led to decrease in disease incidence and severity in double transgenic DR3.DQ8.A β^0 mice as compared to mice treated with isotype control antibody [46]. These set of data clearly indicates that the increased disease severity observed in double transgenic DR3.DQ8.A β^0 mice was due to high levels of IL-17 produced by DQ8 specific T cells. Blocking of IFN γ or IL-17 in DQ8.A β^0 mice by neutralizing antibody did not lead to development of disease.

Pathological analysis of brain and spinal cord tissues of mice with EAE showed that DR3.DQ8.A β^0 double transgenic mice developed severe CNS pathology as compared to DR3.A β^0 transgenic mice. DR3.DQ8.A β^0 mice showed more widespread brain pathology with severe inflammation and demyelination in all parts of the brain tissue, including cerebellum, brain stem, cortex, corpus callosum, stratum, and meninges. In contrast, DR3.A β^0 transgenic mice showed inflammation primarily localized to the meninges of the brain. DR3.DQ8.A β^0 transgenic mice with EAE also showed typical parenchymal white matter loss in brain, the classical pathology observed in MS. A similar pattern of pathology was also observed in the spinal cord with increased inflammation and demyelination in DR3.DQ8.A β^0 mice as compared to DR3.A β^0 mice. Thus, DR3.DQ8.A β^0 double transgenic mice develop severe brain and spinal cord pathology similar to brain pathology observed in human MS.

5. Myelin-specific Antibodies Play an Important Role in PLP₉₁₋₁₁₀ Induced EAE in HLA-DR3DQ8 Transgenic Mice

IL-17 can promote autoimmune disease through a mechanism distinct from its pro-inflammatory effects [47]. It has been linked to the induction of autoreactive humoral immune responses because a deficiency in the blockade of IL-17 results in the decline of the autoantibody response [48]. IL-17 can also induce the formation of germinal centers, leading to the activation of B cells and an increased level of antigen presentation and antibody production. It is possible that the IL-17 induced B cells play an important role in the disease exacerbation of DR3.DQ8.A β^0 mice. Further, administration of anti-myelin antibodies has been shown to enhance demyelination in animal models of MS [49-52]. These pathogenic antibodies have been shown to mediate tissue damage by recruitment of classical complement cascade [52, 53]. The involvement of complement-dependent mechanisms in antibody mediated demyelination and pathogenesis in EAE and MS had been noted in several studies [54, 55]. Therefore, we investigated the role of anti-myelin antibodies and complement in disease exacerbation and the increased CNS pathology observed in DR3DQ8 mice.

We first analyzed levels of anti-myelin specific antibodies in single and double transgenic mice with EAE. DR3.DQ8.A β^0 double transgenic mice and DR3.A β^0 single transgenic mice were immunized with PLP₉₁₋₁₁₀ plus adjuvant as described previously [46] and sera was collected at different time points. Levels of anti-PLP₉₁₋₁₁₀ antibodies were detected using ELISA with PLP₉₁₋₁₁₀ as the capture antigen. As shown in Fig. 1A, DR3.DQ8.A β^0 double transgenic mice with EAE showed higher levels of anti-PLP₉₁₋₁₁₀ antibodies at all time points tested from day 7 to 40 days post immunization. Levels of anti-PLP₉₁₋₁₁₀ IgG increased overtime and maximum levels were observed at day 40 post immunization. Mice immunized with PLP control peptide or CFA alone showed no reactivity (data not shown). We also tested these sera against the whole PLP-MBP molecules (MP4) using a fusion

protein expressing both PLP and MBP protein [36]. Similar to anti-PLP antibodies, we observed that DR3.DQ8.Aβ° double transgenic mice with EAE produced higher levels of anti-MP4 antibodies as compared to DR3.Aβ° single transgenic mice with EAE at all time points (Fig. 1B).

Since, DR3.DQ8.Aβ° double transgenic mice showed severe disease and a higher anti-PLP antibodies level, we hypothesized that increase in clinical and pathological disease might be due to IgG and complement deposition in the CNS leading to tissue destruction. Fresh frozen section were stained with mouse anti-IgG or anti-C3 specific antibody and visualized using fluorescence microscope. As shown in Fig. 2, we observed higher IgG and complement C3 deposition measured in brain sections from DR3.DQ8.Aβ° double transgenic mice with EAE as compared to DR3.Aβ° single transgenic mice with EAE. The deposition was observed in area with inflammatory lesions. We next analyzed expression of C5 mRNA levels in CNS of and observed that the mean C5 mRNA levels in single transgenic DR3 mice were 2 fold higher over control mice. Whereas in DR3.DQ8.Aβ° mice with EAE, C5 mRNA levels were 15 fold higher than control (7.5 fold increased over single transgenic DR3.Aβ° mice with EAE). These data indicate that antibody and complement play a role in inducing the severe pathology observed in double transgenic mice. These findings are in agreement with earlier reports that anti-myelin antibodies and complement might play an important role in inducing CNS demyelination in MS and EAE [49, 52, 56-60]. Antibodies against myelin oligodendrocytes glycoprotein (MOG) had been shown to be present within demyelination lesions of cases with acute MS as well as in the marmoset model of EAE [57]. In addition, adoptive transfer of MBP specific CD4 T cells in combination with demyelination monoclonal antibody specific for MOG in Lewis rats has been shown to induce severe disease associated with large plaques of demyelination [58]. Further, pathogenic anti-myelin antibodies had been shown to induce demyelination through complement activation as mice deficient in C3 develop a milder form of EAE compared to C3 sufficient mice [52, 60].

6. Concluding Remarks

The main advantage of mouse EAE is that genetically engineered mutants can be generated and bred. Thus, the influence of genetics on susceptibility, disease course, inflammation and demyelination can be studied. An EAE mouse model where the autoreactive T cells repertoire is selected and shaped by human MHC class II molecules will provide new information on immunopathogenesis of inflammatory and demyelinating diseases such as human MS. Thus HLA transgenic mice had been quite useful in understanding the role of HLA class II molecule in immunopathogenesis of EAE/MS. The data from our single and double transgenic mice indicate the final outcome of disease might be dependent on interaction between HLA-DR and -DQ molecules. Utilizing these mice we were able to identify immunodominant as well as encephalitogenic epitopes of myelin antigen such as PLP, MBP and MOG. Further, data generated from EAE in HLA class II transgenic mice suggest that HLA-DR molecules is required for susceptibility to disease while the HLA-DQ molecule might play a modulatory role through the pro and anti-inflammatory cytokine network. We have shown that while HLA-DQ6 (DQβ1*0601) can protect DR3 mice from EAE by producing anti-inflammatory IFNγ; HLA-DQ8 (DQβ1*0302) synergize with DR3 to induce a severe disease in DR3DQ8 double transgenic mice by producing pro-inflammatory IL-17 and GM-CSF. Further we have presented data indicating that IL-17 directly or indirectly helps in induction of B cells producing anti-myelin antibodies. We have also shown increased deposition of IgG and complement together with an increased expression of complement in the brain of double transgenic mice with severe EAE. It will be interesting to investigate the mechanism responsible for the production of protective IFNγ from HLA-DQ6 and inflammatory IL-17 from HLA-DQ8 restricted T cells. We join other

contributors of this special issue in recognizing the immense contribution of Chella David for his many achievements in the field of autoimmunity including generation of HLA transgenic mice. Advent of these HLA transgenic mice have helped immensely in understanding the immunopathogenesis of various inflammatory, autoimmune, allergic and infectious diseases. Finally, we note that this issue is part of the Journal of Autoimmunity's commitment in the recognition of outstanding scientists in the field of autoimmunity. We are delighted that Chella Davis is being so honored and that he is part of the continuum of distinguished autoimmunologists and dedicated themes in the journal [61-71].

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Fig. 1. Antibody response against myelin antigens in HLA-class II transgenic mice immunized with PLP₉₁₋₁₁₀ peptide

DR3DQ8 mice with EAE showed higher level of antibody against PLP₉₁₋₁₁₀ peptide (A) as well as whole PLP-MBP fusion protein (B) compared to DR3 mice with EAE at day 7, 20 and 40 post-immunization. Sera were collected at indicated time points and assayed using PLP₉₁₋₁₁₀ or PLP-MBP coated plates using alkaline phosphatase-conjugated (AP) goat anti-mouse IgG (Jackson ImmunoResearch) and p-Nitrophenyl phosphate (PNPP; Southern Biotechnology Associates Inc., Birmingham, AL) as substrate [45].

Fig. 2. Immunofluorescence detection of IgG and complement C3 deposition in brain of DR3 and DR3DQ8 mouse with EAE

DR3DQ8 mice with EAE showed higher expression of IgG (red) and C3 deposition (green) in brain section as compared to DR3 mice with EAE. Expression of IgG (red) and C3 (green) were detected by staining brain section with anti-mouse IgG, anti-C3 antibody and fluorescence conjugate secondary antibody. Sections were fixed, counterstained with nuclear DAPI stain and analyzed using fluorescent microscope.

Fig. 3. The relative mRNA levels of C5 in CNS of DR3.A β ^o single and DR3.DQ8.A β ^o double transgenic mice
 DR3.DQ8.A β ^o showed higher expression of C5 mRNA as compared to DR3.A β ^o mice. Expressions of C5 mRNA in different transgenic mice were quantified by real-time PCR. Expression of β -actin was used as an internal control. The expression of C5 mRNA in CNS of mice immunized with PLP91-110 relative to mice immunized with control PLP peptide was calculated by the $\Delta\Delta C_t$ method.

Fig. 4. Schematic diagram of disease susceptibility and resistance in HLA class II transgenic mice and disease modulation by HLA-DQ molecules

HLA-DR3 transgenic mice recognizing PLP₉₁₋₁₁₀ peptide produce moderate levels of both Th1 (IFN γ) as well as Th17 (IL-17) cytokines and are susceptible to EAE. Whereas DQ6 mice recognizing the same peptide produce high levels of IFN γ and are resistant to EAE. IFN γ produced by DQ6 restricted T cells is anti-inflammatory as it protects DR3DQ6 from development of EAE. In contrast, DQ8 mice recognizing PLP₉₁₋₁₁₀ peptide produce high levels of IL-17 and GM-CSF but are also resistant to development of EAE. Interestingly, presence of DQ8 with DR3 leads to severe disease indicating that DQ8 restricted IL-17 synergizes with DR3 to cause more severe disease. Our recent data show that increased disease and pathology observed in DR3DQ8 double transgenic mice may be due to induction of anti-myelin antibodies by IL-17, which might lead to complement deposition and subsequent neuropathology either through complement mediated cytotoxicity or through antibody dependent cytotoxicity.

Table 1
Human myelin proteolipid protein specific T cell epitopes recognized by HLA class II molecules*

	PLP epitope											
	1-20	31-50	41-60	51-70	81-100	91-110	101-120	139-154	178-197	188-207	208-227	264-277
DR2 (*1502)												
DR3 (*0301)												
DR4 (*0401)												
DQ6 (*0601)												
DQ8 (*0302)												

* only immunogenic region are shown.